Acta Pharmaceutica Sciencia
49: 29-38 (2007)

Synthesis and Evaluation of some Novel Piperidino Thiophenes as Potential Antioxidant and Anti-inflammatory Agents

Dalbir Singh[1*], S. Mohan[2], Prabodh Chander Sharma[1] and J. Sarvanan[2]

[1]Lord Shiva College of Pharmacy, Sirsa-125055, Haryana, INDIA

[2]PES College of Pharmacy, Bangalore-560050, Karnataka, INDIA

Abstract

A series of piperidino thiophenes was synthesized with an objective to develop novel and potent antioxidant and anti-inflammatory agents of synthetic origin. First, p-fluoro aniline and ethyl cyano acetate were reacted to yield p-fluoro cyano acetanilide (1). Then, compound (1) was reacted with N-methylpiperdin-4-one to obtain an intermediate, which was processed to 2-amino-3-(p-fluorocarboxanilido)-6-methyl piperidino (4, 3-b) thiophene (2) by the well known and versatile Gewald reaction. Reaction of compound (2) with different aromatic aldehydes yielded the title compounds (3a-3m). The synthesized compounds were purified, characterized and evaluated for their antioxidant and anti-inflammatory activities. Most of the compounds exhibited moderate to significant activities.

Key Words: Piperidino thiophenes, Schiff bases, Gewald reaction, anti-inflammatory activity, antioxidant activity

Introduction

A number of thiophenes and schiff bases have been reported to possess significant and diverse biological activities such as antifungal (Ryu *et al.*, 2005; Govindaswamy and Mohan, 1998), analgesic (Shafeeque *et al.*, 1999), anti-inflammatory (Kumar *et al.*, 2004; Pillai *et al.*, 2004; Raju *et al.*, 1998; Laddi *et al.*, 1998), antibacterial (Dzhuravey *et al.*, 1992), antioxidant (Ferreira *et al.*, 2006), antitumor (Jarak *et al.*, 2005), local anesthetic (Gadad *et al.*, 1994) and antimicrobial activities (Ferreria *et al.*, 2004; Saravanan and Mohan, 2003). On the other hand radicals and retard the progress of many chronic diseases such as vascular diseases, oxidative stress responsible for DNA, protein and membrane

* Corresponding author e-mail: sheorandalbir@yahoo.co.in

damage and some form of cancer (Ferreira *et al.*, 2006; Nakayama *et al.*, 1993). Hence, in the light of above findings and as a part of our ongoing programme on synthesis and evaluation novel therapeutic agents with anti-inflammatory and antioxidant properties, a number of thiophenes and schiff basis with significant biological activities have been prepared in our laboratories (Shafeeque *et al*, 1999; Mohan *et al.*, 1997; Raju *et al.*, 1998; Govindaswamy and Mohan, 1998; Saravanan and Mohan, 2003). So, in continuation to these efforts and with an objective to develop novel and potent therapeutic agents of synthetic origin, it was decided to synthesize certain 2-substituted-3-(p-fluorocarboxanilido)-6-methyl piperidino (4, 3-b) thiophene and evaluate them for their antioxidant and anti-inflammatory potential.

Materials and Methods

The melting points of synthesized compounds were determined in open capillary tubes using Veego VMP-1 melting point apparatus, expressed in ^{0}C and are uncorrected. The IR spectra of compounds were recorded on Perkin Elmer Infra Red Spectrophotometer in KBr disc and absorption bands are expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Brucker Aveance 700 MHz NMR Spectrometer (Chemical shift if δ ppm) using TMS as internal standard. Absorbance was recorded on a Shimadzu UV-1602 double beam spectrophotometer and is expressed in nm. Reactions were monitored by thin layer chromatography on pre-coated plates using different solvent systems. The purity of synthesized compounds was ascertained by TLC, using iodine vapors as visualizing agents.

Chemistry

The title compounds were prepared in following steps:

4-fluorocyanoacetanilide(1)

A mixture of 4-fluoroaniline (1.0 mol) and ethyl cyano acetate (1.0 mol) was heated on an oil bath at 160-170^{0}C for 6 h. The reaction mixture was left overnight at room temperature. The solid, thus obtained was washed with ethanol, dried and recrystallized.

2-amino-3-(p-fluorocarboxanilido)-6-methyl piperidino (4, 3-b) thiophene (2)

A mixture of p-fluoro cyano acetanilide(1) (0.04mol), N-methyl piperidin-4-one (0.04mol), ammonium acetate (2g) and glacial acetic acid (2ml) in benzene (100ml) was refluxed for 8 h in Dean Stark apparatus, with an arrangement for continuous separation of water. After 8 h the reaction mixture was cooled, diluted with 10 ml benzene and washed with sodium carbonate solution (10% w/v in water) and water successively and dried over anhydrous sodium sulphate. The solvent was removed under vacuum. The intermediate crude product obtained was immediately processed by reacting with sulfur (1.28 g) in alcohol (30 ml) at 45-50 ^{0}C adding diethyl amine (4 ml) drop wise with continuous stirring for 3h to yield 2-amino-3-(p-fluorocarboxanilido)-6-methyl piperidino (4, 3-b) thiophene (2). The reaction

mixture was chilled over night and the solid obtained was filtered, washed with ethanol and crystallized from benzene.

Synthesis of Schiff bases (3a-3m)

A mixture of 2-amino-3-(p-fluorocarboxanilido)-6-methyl piperidino (4, 3-b) thiophene (2) (0.05mol) and appropriately substituted aryl aldehyde (0.05mol) was reacted in ethanol (in presence of catalytic amount of glacial acetic acid) by heating under reflux for 3h. The solid product obtained was filtered washed with ethanol, dried and recrystallized. Physical and analytical data of synthesized compounds is summarized in Table-1 and characterization data inTable-2.

Table 1. Physical and analytical data of synthesized compounds

Comp. No.	R	M.P. (^{0}C)	Yield (%)	Molecular Formula	Molecular Weight	R_f value	R_m Value
1	-	172	51	$C_9H_7FN_2O$	178	0.41	-0.15
2	-	160	48	$C_{15}H_{16}FN_3OS$	305	0.49	-0.017
3a	4-OH	268	61	$C_{22}H_{20}FN_3O_2S$	409	0.61	-0.20
3b	2-NO_2	257	54	$C_{22}H_{19}FN_4O_3S$	438	0.43	-0.120
3c	3-NO_2	251	52	$C_{22}H_{19}FN_4O_3S$	438	0.48	-0.033
3d	2-OH	246	54	$C_{22}H_{20}FN_3O_2S$	409	0.35	-0.267
3e	2-Cl	238	48	$C_{22}H_{19}ClFN_3OS$	428	0.68	-0.327
3f	4-OH, 3-OMe	262	58	$C_{23}H_{22}FN_3O_3S$	439	0.72	-0.420
3g	4-OMe	221	52	$C_{23}H_{22}FN_3O_2S$	423	0.64	-0.251
3h	3,4-di-OMe	235	61	$C_{24}H_{24}FN_3O_3S$	453	0.63	-0.23
3i	4-N(Me)$_2$	218	65	$C_{24}H_{25}FN_4OS$	436	0.57	-0.124
3j	3,4,5-tri-OMe	253	63	$C_{25}H_{26}FN_3O_4S$	483	0.52	-0.034
3k	4-Cl	224	42	$C_{22}H_{19}ClFN_3OS$	428	0.42	-0.013
3l	H	175	60	$C_{22}H_{20}FN_3OS$	393	0.69	-0.356
3m	4-Me	207	52	$C_{23}H_{22}FN_3OS$	407	0.58	-0.142

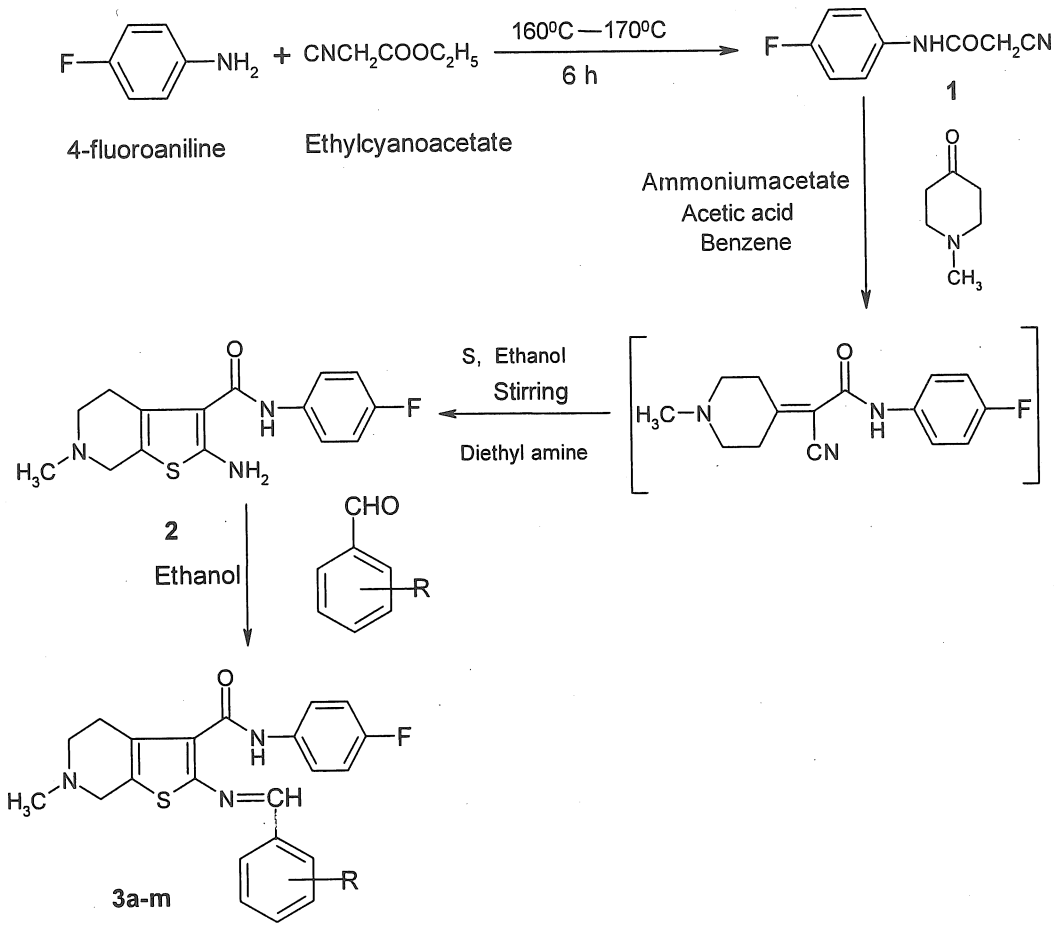
Biological Screening

Antioxidant Screening

Antioxidant activity was carried out by reduction method where increase in absorbance of the reaction mixture indicates the reducing power of the samples (Khanam *et al.* 2004). Test compounds were mixed with phosphate buffer and potassium ferricyanide [$K_3Fe(CN)_6$] (1%) and the mixture was incubated at 50°C for 30 minutes. Then, trichloro acetic acid was added to the mixture, and the same was then centrifuged at 3000 rpm for 10 minutes. Finally, upper layer was

separated, mixed with distilled water and ferric chloride (0.1%) and the absorbance was recorded at 700 nm. Ascorbic acid was taken as a standard for antioxidant activity.

Scheme:



Anti inflammatory Screening

Anti-inflammatory activity screening was carried out by inhibition of bovine serum albumin denaturation method (Elias and Rao, 1988) using Ibuprofen as a standard. The test compounds were dissolved in minimum amount of water and diluted with

phosphate buffer (0.2M, pH 7.4). Test solution containing different concentrations of drug was mixed with albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$ C for 15 minutes. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$C in a water bath for 10 minutes. After cooling, the turbidity of the resulting solution was measured at 660 nm. Each experiment was done in triplicate and the average reading was taken. The results of biological screening are summarized in Table-3.

Results and Discussion

In the present study we report synthesis of 13 novel schiff bases. Compound (1) was synthesized by reacting p-fluoro aniline and ethyl cyano acetate. The IR Spectrum of the compound (1) shows (C≡N) peak at 2310 cm^{-1}, which is absent in compound (2). The compound (2) shows distinct peaks at 3370 cm^{-1} (NH_2); 825 cm^{-1} (C-N). Substantial proof for the formation of these new compounds has been provided by differences in their ^1H NMR spectra, melting points and R_f values from that of parent compound and each other. The formation of schiff's bases (3a-m) was also confirmed from the IR spectrum of the compounds. The absence of 3370 cm^{-1} (NH_2) and presence of IR peak at near by 1550 cm^{-1} indicated the -N=CH- peak. Compound 3f, with a hydroxy and methoxy substitution was found to posess most potent anti-inflammatory activity followed by compound 3d and 3a having a hydroxy substitution at ortho and para positions. It indicates that anti-inflammatory activity may be associated with electron donating capacity of substituents. Compound 3f exhibited most potent antioxidant activity followed by 3a and 3e. Mass spectra of Compound 3i recorded in Maldi MS showed m/z peak at 436.

Table 2. Characterization data of synthesized compounds

Comp No.	λ_{max} in ethanol (nm)	IR (KBr, CM^{-1})	^1H NMR (δ ppm, $CDCL_3$/TMS)
1	208.0	3279 (-NH-); 2310 (C≡N); 3107 (Ar-CH); 1670 (C=O); 1220 (C-F).	
2	245.0	3370 (NH2); 3247 (-NH-); 2929 (CH-Ar); 1667 (C=O); 1212 (C-F) ; 825 (C-N).	
3a	370.5; 245; 208.5	3315 (OH); 3239 (-NH-); 2938 (CH-Ar); 1669 (C=O); 1539 (-N=CH); 1170 (C-F); 820 (CN).	----------
3b	371; 245.5; 210.5	3310 (-NH-); 1653 (C=O); 1521 (-N=CH); 1538,1320 (NO_2); 1212 (C-F); 837 (C-N).	10.94 (s, 1H, NH); 8.89(s, 1H, N=CH); 8.51 (d, 1H, CH); 7.76 (q, 2H,CH); 7.61 (d, 1H, CH); 7.55 (t, 1H, CH); 7.41 (t, 1H, CH); 7.12 (q, 2H, CH); 3.62 (s, 2H, CH_2); 3.23 (t, 2H, CH_2); 2.73 (t, 2H, CH_2); 2.56 (s, 3H, CH_3).
3c	356; 242.5; 211.5	3218 (-NH-); 1658 (C=O);1521 (-N=CH); 1532,1365 (NO_2); 1211 (C-F) 839 (C-N).	----------
3d		3315 (OH); 3239 (-NH-); 2938 (CH-Ar); 1669 (C=O); 1539 (-N=CH); 1170 (C-F); 820 (CN)	----------
3e	370.5;249.5;208.5	3254 (-NH-); 2920 (CH-Ar); 1672 (C=O); 1540 (-N=CH); 1225 (C-F); 829 (CN); 780 (C-Cl).	10.93 (s, 1H, NH); 8.91(s, 1H, N=CH); 8.28 (d, 1H, CH); 7.73 (q, 2H,CH); 7.59 (d, 1H, CH); 7.50 (t, 1H, CH); 7.40 (t, 1H, CH); 7.10 (q, 2H, CH); 3.62 (s, 2H, CH_2); 3.22 (t, 2H, CH_2); 2.78 (t, 2H, CH_2); 2.52 (s, 3H, CH_3).
3f	399.5; 233.5; 211	3278 (OH); 3226 (-NH-); 2935 (CH-Ar); 1669 (C=O); 1538 (-N=CH); 1222 (C-F); 831 (CN)	----------

Table 2. Characterization data of synthesized compounds (Cont…)

3g	387; 249; 209	3249 (-NH-); 1660 (C=O); 1522 (-N=CH); 1222 (C-F); 828 (CN)	----------
3h	395; 250; 208	3260 (-NH-); 2941 (CH-Ar); 1672 (C=O); 1540 (-N=CH); 1225 (C-F); 825 (CN)	11.10 (s, 1H, NH); 8.38 (s, 1H, CH); 7.65 (q, 2H, CH); 7.46 (s, 1H, CH); 7.38 (d, 2H, CH); 7.02 (q, 2H, CH); 4.0 (s, 3H, OCH_3); 3.90 (s, 3H, OCH_3); 3.62 (t, 2H, CH_2); 3.23 (t, 2H, CH_2); 2.74 (t, 2H, CH_2); 2.51 (s, 3H, CH_3)
3i	449.5; 253; 206.5	3232 (-NH-); 2924 (CH-Ar); 1664 (C=O); 1526 (-N=CH); 1181 (C-F); 823 (CN)	11.42 (s, 1H, NH); 8.30 (s, 1H, CH); 7.76 (d, 2H, CH); 7.65 (q, 2H, CH); 7.08 (q, 2H, CH); 6.76 (d, 2H, CH); 3.68 (s, 2H, CH_2); 3.27 (t, 2H, CH_2); 2.83 (t, 2H, CH_2); 3.11 (s, 6H, $N(CH_3)_2$); 2.54 (s, 3H, N-CH_3)
3j	386; 248.5; 211	3249 (-NH-); 2922 (CH-Ar); 1662 (C=O); 1521 (-N=CH); 1227 (C-F); 818 (CN)	----------
3k		3260 (-NH-); 1669 (C=O); 1540 (-N=CH); 1225 (C-F); 825 (CN); 772 (C-Cl).	----------
3l	367; 254; 209	3238 (-NH-); 2936 (CH-Ar); 1677 (C=O); 1508 (-N=CH); 1216 (C-F); 847 (CN)	----------
3m	380; 249.5; 213	3232 (-NH-); 2933 (CH-Ar); 1667 (C=O); 1538 (-N=CH); 1208 (C-F); 845 (CN)	----------

Table 3. Anti-inflammatory and antioxidant activity of synthesized compounds

Comp. No.	Anti-inflammatory activity (% Bovine serum inhibition)*	Antioxidant Activity (%)
3a	51.35	52.70
3b	27.58	19.77
3c	30.38	21.11
3d	53.24	35.82
3e	36.61	52.45
3f	55.18	79.69
3g	30.27	21.58
3h	35.46	45.68
3i	32.52	39.78
3j	38.92	47.62
3k	40.16	37.59
3l	27.84	16.83
3m	29.64	18.16
Ibuprofen	68.22	----
Ascorbic Acid	-----	100

*Results average of three readings

References

Dzhuravey, A.D., Karimkulov, K.M. and Makhsumov, A.G., (1992). Antibacterial activity of new thiophenes derivatives. *Khim-farm. Zh.* 26 (11012):73-75.

Ferreira, C.F.R., Maria-Joao, R.P., Vilas-Boas, M., Estevinho, L.M., Begouin, A. and Gilbert, K., (2006). Evaluation of the antioxidant properties of diarylamines in the benzo[*b*]thiophene series by free radical scavenging activity and reducing power. *Bioorg. Med. Chem. Lett.* 16:1384-1387.

Ferreira, I.C.F., Calhelha, R.C., Estevinho, L.M. and Queiroz, M.J.R., (2004). Screening of antimicrobial activity of diaryl amine in the 2,3,5- trimethyl benzo[*b*]thiophene series: a structure-activity evaluation study. *Bioorg. Med. Chem. Lett.* 14:5831-5833.

Gadad, A.K., Kumar, H., Shishoo, C.J., Mkhazi, I. and Mahajanshetti, C.S., (1994). Synthesis of some 2-aminoacetylamino-3-carbethoxy/anilido-4,5,6,7-tetrahydro-benzo[*b*]thiophenes for local anesthetic activity. *Ind. J. Chem. Soc.* 33:298-301.

Govindaswamy, P. and Mohan, S., (1998). Synthesis of 2-substituted 5,6-dimethyl thieno (2,3-d)3, 1-oxazin-4-one for antifungal activity. *Ind. J. Heterocyclic Chem.* 7:205-208.

Elias, G. and Rao, M.N.A., (1988). Inhibition of albumin denaturation and anti-inflammatory activity of dehydrozingerone and its analogs. *Indian J. Experiment. Biol.* 26:540-542.

Jarak, I., Kralj, M., Suman, L., Pavlovic, G., Dogan, J. and Piantanida, I., (2005). Novel cyano- and N-isopropylamidino-substituted derivatives of benzo[b]thiophene-2-carboxanilides and benzo[b]thieno[2,3-c]quinolones : Synthesis, photochemical synthesis, crystal structure determination, and antitumor evaluation. *J. Med. Chem.* 48:2346-2360.

Khanam, S., Shivaprasada, H.N. and Devi, K., (2004). *In vitro* antioxidant screening models: A review. *Indian J. Pharm. Edu. Res.* 38(4): 180-183.

Kumar, P.R., Raju, S., Goud, P.S., Sailaja, M., Sharma, M.R. and Reddy, G.O., (2004). Synthesis and biological evaluation of thiophenes [3,2-*b*] Pyrrole derivatives as potential anti-inflammatory agents. *Bioorg. Med. Chem.* 12:1221-1230.

Laddi, U.V., Talwar, M.B., Desai, S.R., Somannavar, Y.S., Bennur, R.S. and Bennur, S.C., (1998). Anti-inflammatory activity of 3-substituted-4-amino-5-piperidino-4-(*H*)-1,2,4-triazoles. *Indian Drugs* 35(8):509-512.

Mohan, S., Saravanan, J., Nargund, L.V.G. and Shishoo, C.J., (1997). Synthesis of some benzo(*b*)thiophenes as a potential antimicrobial agents. *Indian J. Heterocyclic Chem.* 6:203-206.

Nakayama, T., Yamada, M., Osawa, T. and Kawakishi, S., (1993). Supression of active oxygen-induced cytotoxicity by flavonoids. *S. Biochem. Pharmacol.* 45:265-267.

Pillai, A.D., Rathod, P. D., Franklin, X. P., Kamala, V. K., Harish, P. and Vasudevan, S., (2004). Design, synthesis, and pharmacological evaluation of some 2-[4-morphplino]-3aryl-5-substituted thiophenes as novel anti-inflammatory agents: generation of a novel anti-inflammatory pharmacophore. *Bioorg. Med. Chem. Lett.* 12:4667-4671.

Raju, V.S., Mohan, S. and Saravanan, J., (1998). Synthesis of 2-substituted-amino-3-(N-o-tolylcarboxamido)-4,5-dimethyl thiophenes as analgesic and anti-inflammatory agents. *Asian J.Chem.* 8:59-62.

Ryu, C.K., Lee, S.K., Han, J.Y., Jung, O.J., Lee, J.Y. and Jeong, S.H., (2005). Synthesis and antifungal activity of 5-arylamino-4,7-dioxobenzo[*b*] thiophenes. *Bioorg. Med. Chem. Lett.* 15:2617-2620.

Saravanan, J. and Mohan, S., (2003). Synthesis of some 2-amino-3-(N-tolyl carboxamido)-4,5-pentamethylene thiophenes as potential antibacterial agents. *Asian J. Chem.* 15(2):625-628.

Shafeeque, S., Mohan, S. and Manjunatha, K.S., (1999). Synthesis, analgesic and anti-inflammatory activity of some 2-substituted amino-3-(N-p-tolyl carboxamido)-4,5-dimethyl thiophenes. *Indian J. Heterocyclic Chem.* 8(4):297-300.

Received: 13.07.2006

Accepted: 27.11.2006